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Simultaneous spectrophotometric determination of diazepam and propranolol hydrochloride in tablets

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of diazepam and propranolol hydrochloride in combined tablet dosage form. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. Diazepam and propranolol hydrochloride show an isoabsorptive point at 235 nm in 0.05 M methanolic sulphuric acid. The second wavelength used is 215 nm, which is the λ-max of propranolol hydrochloride in 0.05 M methanolic sulphuric acid. The linearity was obtained in the concentration range of 1-20 µg/ml for both diazepam and propranolol hydrochloride. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ-max of propranolol hydrochloride. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found. The results of analysis have been validated statistically and by recovery studies.

Keywords: Diazepam, Propranolol hydrochloride, Absorbance ratio method, Spectrophotometric, Tablet, Validation

1. INTRODUCTION

Diazepam (DZP) (Figure 1) is chemically 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-1, 4-benzodiazepin-2-one; $C_{16}H_{13}ClN_2O$[1], used as an anxiolytic agent[2]. It is official in IP, USP and BP. IP[3] and BP[4] describes non-aqueous titration method and USP[5] describe liquid chromatography method for its estimation. Literature survey reveals spectrophotometric[6-9], spectrofluorimetric[10], GC[11], HPLC[12], HPTLC[13-14], LC/MS[15] and radioimmunoassay[16] methods for the estimation of DZP in single dosage form. Propranolol hydrochloride (PRO) (Figure 2) is chemically (*RS*)-1-isopropylamino-3- (1-naphthyloxy) propan-2-ol hydrochloride; $C_{16}H_{21}NO_2$, HCl[17], is beta-adrenoceptor antagonist[18]. The combination of DZP and PRO has been shown to be effective in the management of chronic anxiety. The combination was generally more effective than diazepam[18]. Propranolol hydrochloride is official in IP, USP and BP. IP[19] and BP[20] describes potentiometric titration method and USP[21] describe liquid chromatography method for its estimation. Literature survey reveals spectrophotometric[22-24], fluorimetric[25], HPLC[26-27] and chemiluminescence[28] methods for estimation of propranolol hydrochloride in single dosage form. This combination is not official in any pharmacopoeia, so no official method is available for the estimation of these two drugs in combined dosage forms. Literature survey reveals spectrophotometric[29] method for the simultaneous estimation of DZP and PRO in combined dosage form. The present manuscript describes simple, sensitive accurate, precise, rapid and economic first order derivative spectrophotometric method for simultaneous determination of diazepam and propranolol hydrochloride in pharmaceutical tablet dosage form.

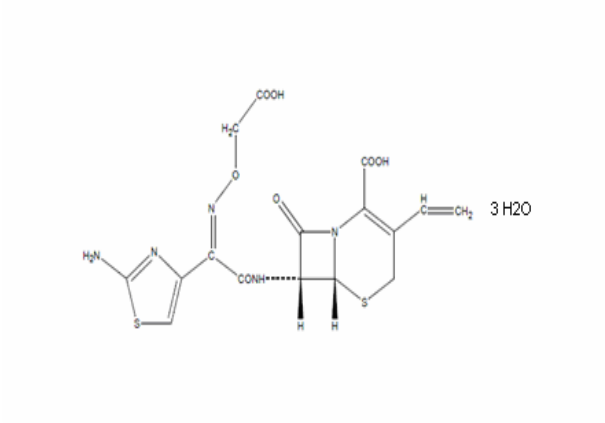

Figure 1: Chemical structure of diazepam (DZP)

2. MATERIALS AND METHODS

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

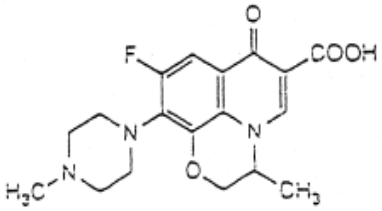

Figure 2: Chemical structure of propranolol hydrochloride (PRO)

Reagents and materials

DZP and PRO bulk powder was kindly gifted by Santham Pharmaceutical Ltd, Gandhinagar, Gujarat, India. The commercial fixed dose combination product containing 2 mg DZP and 10 mg PRO was procured from the local pharmacy. Methanol and sulphuric acid, AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India.

Preparation of diluent, standard and sample solutions

Preparation of 0.05 M Methanolic Sulphuric Acid

0.05 M methanolic sulphuric acid was prepared by adding 2.7 ml concentrated sulphuric acid in 1000 ml volumetric flask and diluting up to the mark with methanol.

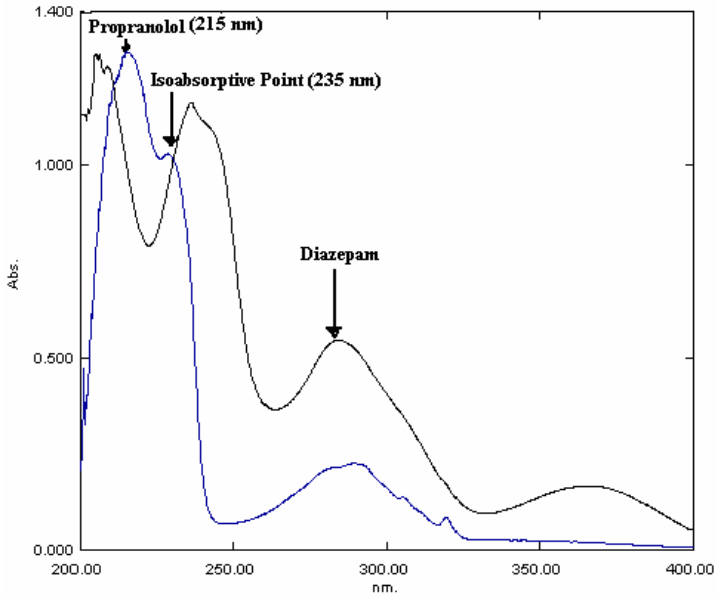

Figure 3: Overlain absorption spectra of DZP (298 nm) and PRO (215 nm) showing isoabsorptive point (235 nm) in 0.05 M methanolic sulphuric acid

Preparation of standard solutions

Accurately weighed DZP (10 mg) and PRO (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with 0.05 M methanolic sulphuric acid to obtain a standard solutions having concentration DZP (100 µg/ml) and PRO (100 µg/ml).

Preparation of sample solution

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 2 mg of DZP and 10 mg of PRO was transferred to a 100 ml volumetric flask. The content was mixed with 0.05 M methanolic sulphuric acid (50 ml), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was filtered through a Whatman filter paper No. 41. The volume was adjusted up to the mark with 0.05 M methanolic sulphuric acid. An aliquot of this solution (0.5 ml) was taken in to a 10 mL

Table No. 1. Recovery data for the proposed method

* Mean % Recovery ± SD of five observations

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (%)	Mean% Recovery ± SD*
DZP	I	1	50	100.6 ± 1.35
	II	1	100	100.6 ± 1.00
	III	1	150	100.7 ± 1.56
PRO	I	5	50	100.1 ± 0.23
	II	5	100	100.8 ± 0.45
	III	5	150	100.3 ± 0.33

volumetric flask and the volume was adjusted up to mark with 0.05 M methanolic sulphuric acid.

Determination of the analytical wavelengths

The standard solutions of DZP (10 µg/ml) and PRO (10 µg/ml) were scanned separately in the UV range of 200 - 400 nm. Data were recorded at an interval of 1 nm. From the overlain spectra of the drugs, two analytical wavelengths i.e. 235 nm (isoabsorptive point) and 215 nm (λ_{max} of PRO) were selected and absorbances were measured at these selected wavelength.

Methodology

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that DZP and PRO show an isoabsorptive point at 235 nm. The second wavelength used is 215 nm, which is the λ-max of PRO. Six working standard solutions having concentration 1, 4, 8, 12, 16 and 20 µg/ml for DZP and PRO were prepared in 0.05 M methanolic sulphuric acid, and the absorbances at 235 nm (isoabsorptive point) and 215 nm (λ-max of PRO) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations

$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1/aX_1 \quad \text{.....(1)}$$

$$C_Y = (A_1/aX_1) - C_X \quad \text{.....(2)}$$

Where, A_1 and A_2 are absorbances of mixture at 235 nm and 215 nm; and aX_1 and aY_1 are absorptivities of DZP and PRO at 235 nm; aX_2 and aY_2 are absorptivities of DZP and PRO respectively at 215 nm; and $Q_M = A_2 / A_1$, $Q_X = aX_2 / aX_1$ and $Q_Y = aY_2 / aY_1$.

Validation of the proposed method[30]

Calibration curve (linearity)

Calibration curves were plotted over a concentration range of 1 – 20 µg/mL for DZP and PRO. Accurately measured standard working solutions of DZP and PRO (0.1, 0.4, 0.8, 1.2, 1.6 and 2.0 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with 0.05 M Methanolic sulfuric acid, and the absorbance was measured at 215nm (λ_{max} of Propranolol) and at 235nm (isoabsorptive point). The calibration curves were constructed by plotting absorbances Vs concentrations.

Table No. 2. Analysis of DZP and PRO by proposed method

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 3)	
	DZP	PRO	DZP	PRO)	DZP	PRO
I	200	200	199.4	197.9	99.68 ± 0.84	98.95 ± 1.47
II	200	200	202.4	198.6	101.2 ± 1.25	99.32 ± 0.97

Accuracy (% recovery)

The accuracy of the method was determined by calculating recoveries of DZP and PRO by the standard addition method. Known amounts of standard solutions of DZP and PRO were added at 50, 100 and 150 % levels to prequantified sample solutions of DZP and PRO. The amounts of DZP and PRO were estimated by applying the obtained values to the above equations.

Method precision (% repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of DZP and PRO (8 µg/mL) without changing the parameters for

Table No. 3. Regression analysis data and summary of validation parameters

Parameters	Absorbance ratio method		
	DZP at 215 nm	PRO at 215 nm	DZP and PRO at 235 nm
Concentration range (µg/ml)	1 – 20	1 – 20	1 – 20
Slope	0.0233	0.0587	0.0347
Intercept	0.0061	0.0357	0.0446
Correlation coefficient	0.9971	0.9972	0.9978
LOD[a] (µg/mL)	0.12	0.05	0.07
LOQ[b] (µg/mL)	0.36	0.14	0.21
Repeatability (% RSD[c], n[d] = 6)	0.54 %	0.16 %	0.32 %
Precision (% RSD)			
Interday (n = 6)	0.47 – 1.93 %	0.28 – 2.02 %	0.47 – 1.90 %
Intraday (n = 6)	0.43 – 1.50 %	0.10 – 1.00 %	0.19 –1.10 %

[a] LOD = Limit of detection [b] LOQ = Limit of quantitation

[c] RSD = Relative standard deviation [d] n = number of determinations

the Q-Absorbance ratio method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of DZP and PRO (4, 8 and 12 µg/mL). The results were reported in terms of relative standard deviation (RSD).

Limit of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline[30].

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

Analysis of DZP and PRO in combined dosage form

Pharmaceutical formulation of DZP and PRO was purchased from local pharmacy. Sample solution was prepared as described earlier. This solution was then analyzed to obtain the spectra and absorbance values at 215 nm and at 235 nm were noted. These values were then equated in equation 1 and 2 mentioned above and the concentrations of each drug were calculated.

3. RESULTS AND DISCUSSION

In the Q-Absorbance ratio method, the absorbance was measured at two wavelengths, one being the isoabsorptive point of the two components and other being the wavelength of maximum absorption of one of the two components. For this measurement, the solutions of DZP and PRO were prepared separately in 0.05 M methanolic sulphuric acid at a concentration of 10 µg /mL. They were scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 1 nm. From the overlain spectra of the two drugs (Figure 3) absorbances were measured at selected wavelength i.e. 235 nm isoabsorptive point and 215 nm, λ_{max} of Propranolol. The absorbance and absorptivity values at the particular wavelengths were calculated and substituted in the respective equations mentioned above to obtain the concentration.

The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1). The method was successfully used to determine the amounts of DZP and PRO present in the tablet dosage forms. The results obtained were in good agreement with the corresponding labeled amount (Table 2). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is given in Table 3. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in combined dosage form.

4. CONCLUSION

The method was found to be simple, sensitive, accurate, precise and cost effective and can be used for the routine quality control analysis of DZP and PRO in combined dosage form without any interference of excipients.

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